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TAIL MELANOPHORES OF XENOPUS IN NORMAL DEVELOPMENT AND REGENERATION [1]

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A peculiar group of light-sensitive melanophores occurs in the ventral fin of Xenopus larvae. Bles (1905) noticed that these chromatophores are contracted during the day, but expand at night, darkening the tail markedly. More recently, Bagnara (1957) has shown that the melanophores of the tail fin are directly photosensitive and refers to this response as the "tail darkening reaction." His suggestion that a photochemical system is involved has been supported by Van der Lek, De Heer, Burgers and von Oordt (1958) who point out that the degree of melanophore response is a function of light intensity.

Because of the above observations and in view of the report that the numerical density of tail fin melanophores is only slightly influenced by the hypophysis (Bagnara, 1957), it seems likely that the presence or absence of light, not the pituitary, is the primary effector of tail melanophore response. The chromatotrophic hormone (CTH) of the hypophysis exerts some influence on these cells, however, as was shown by Thing (1952) who used the tail fin melanophores for assay of such hormone preparations.

It is curious that these melanophores are restricted to the distal half of the ventral fin (Bles, 1905; Bagnara, 1957). Apparently, the tip of the tail provides an environment favorable not only for the establishment of a light-sensitive mechanism, but also for melanogenesis. That the caudal portion of the ventral fin is generally chromogenic is suggested by our previous observation (Bagnara, 1957) that guanophores, which are never present in the tail of normal Xenopus larvae, develop in the distal portion of such tadpoles which have been deprived of their hypophyses.

The present study concerns the responses of tail fin melanophores of Xenopus to changes in illumination during the course of normal development and regeneration, and compares these melanophores with those in other areas of the larvae.

Materials and Methods

The larvae of *Xenopus laevis* used in this investigation were reared from eggs obtained from natural spawning of our adult stock which are kept in outdoor tanks.

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In a few cases, however, ovulation was induced with chorionic gonadotrophic hormone. Throughout the larval period, powdered nettle was employed for feeding. Experiments were carried out in a temperature-controlled room at temperatures of 21° C. during the spring and summer and 24° C. during the fall and winter. Illumination was from north-facing windows.

During some of the experiments, larvae were subjected to darkness for periods of thirty minutes to several hours. Thirty minutes is approximately the minimal time interval for achievement of the tail darkening reaction (Bagnara, 1957; Van der Lek *et al.*, 1958). Dark treatment was carried out in a closed drawer in a darkened room. After appropriate intervals of exposure to darkness, the larvae were immersed immediately in 25% formalin. Such treatment insures rapid fixation and prevents melanophore contraction during the fixing process. The same method of fixation was employed in all experiments in which degree of expansion was to be observed. For long-term preservation, the larvae were removed to 10% formalin.

For the tail regeneration experiments, young Xenopus larvae of stages 51 through 54 (stages of Nieuwkoop and Faber, 1956) were used because older animals usually conclude their metamorphosis before tail regeneration is completed. Tails were cut off with iridectomy scissors while the larvae were hovering in a stationary position. The cuts were made just anterior to the pigmented area. In this way, enough tail remained to allow the larvae sufficient mobility for feeding and for respiration. Care was taken to use only tadpoles on which none of the pigmented area of the fin remained.

In order to evaluate the degree of melanophore response, the melanophore index (M. I.) of Hogben and Slome (1931) was employed. This index is general enough to permit analysis even of types of melanophores which differ slightly from one another in form.

Results

I. Normal development of the fin melanophores

Tail fin melanophores begin to appear at stage 47 (Fig. 1). They are first seen immediately adjacent to the somitic area at the very tip of the tail. The pigmented region gradually increases in size so that at stage 51 the distal third of the tail fin contains many melanophores. By stage 52 the pigmented area has increased to cover the entire caudal half of the tail. The anterior boundary of the pigmented region forms a clear line of separation from the proximal portion of the ventral fin which contains no melanophores at all.

In their early developmental stages, the young tail melanophores are elongated and sparsely branched. As differentiation proceeds, new branches are added radially so that the expanded melanophores appear stellate. During stages of expansion, melanophores of the tail fin differ from those of the head and trunk in that their processes are thin and much more heavily branched. As tail pigmentation proceeds during the early larval period, new melanophores are added at the edge and tip of the fin. Actually, there is a gradient in degree of differentiation; thus, under expanded conditions, melanophores immediately adjacent to the somites are fully developed and stellate while those at the edge are elongated (Fig. 2). Between these two areas exist partially differentiated melanophores

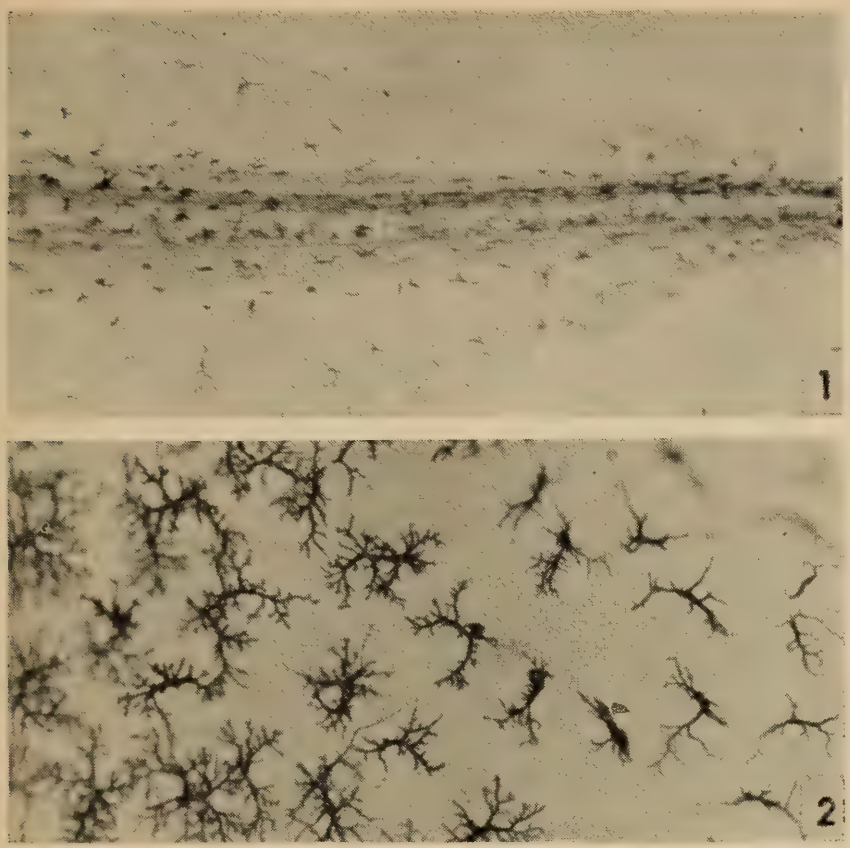

FIGURE 1. First appearance of tail fin melanophores at stage 47. Normal aquarium illumination. Magnification: × 10.

FIGURE 2. Ventral fin of stage 53 larva during the tail darkening reaction. Melanophore differentiation gradient from somites at left to fin edge at right can be seen in these expanded melanophores. Magnification: × 50.

with an intermediate number of branches. This suggests either that new melanophores are proliferating at the edge of the fin or that here unpigmented melanocytes are coming under the influence of a melanogenic substance which is distributed along a gradient from the somites to the ventral fin edge.

II. Normal development of the tail darkening reaction

Although not as striking as that of fully differentiated melanophores, the response of differentiating melanophores is discernible almost from their first appearance. Under normal room illumination, young melanophores are not able to assume the punctate form of the fully developed tail melanophores (Fig. 3); instead, they appear elongate with relatively few visible branches (Figs. 1 and 2). After exposure to darkness for thirty minutes or more, however, their branches become more obvious and often secondary branches can be seen (Fig. 2). Fully formed tail melanophores seen during the tail darkening reaction are stellate and possess secondary and tertiary branches (Fig. 4).

III. Regeneration of tail melanophores and the tail darkening reaction

Approximately four days after tail amputation, a new fin can be seen forming at the end of the regeneration blastema and at the cut edge of the ventral and

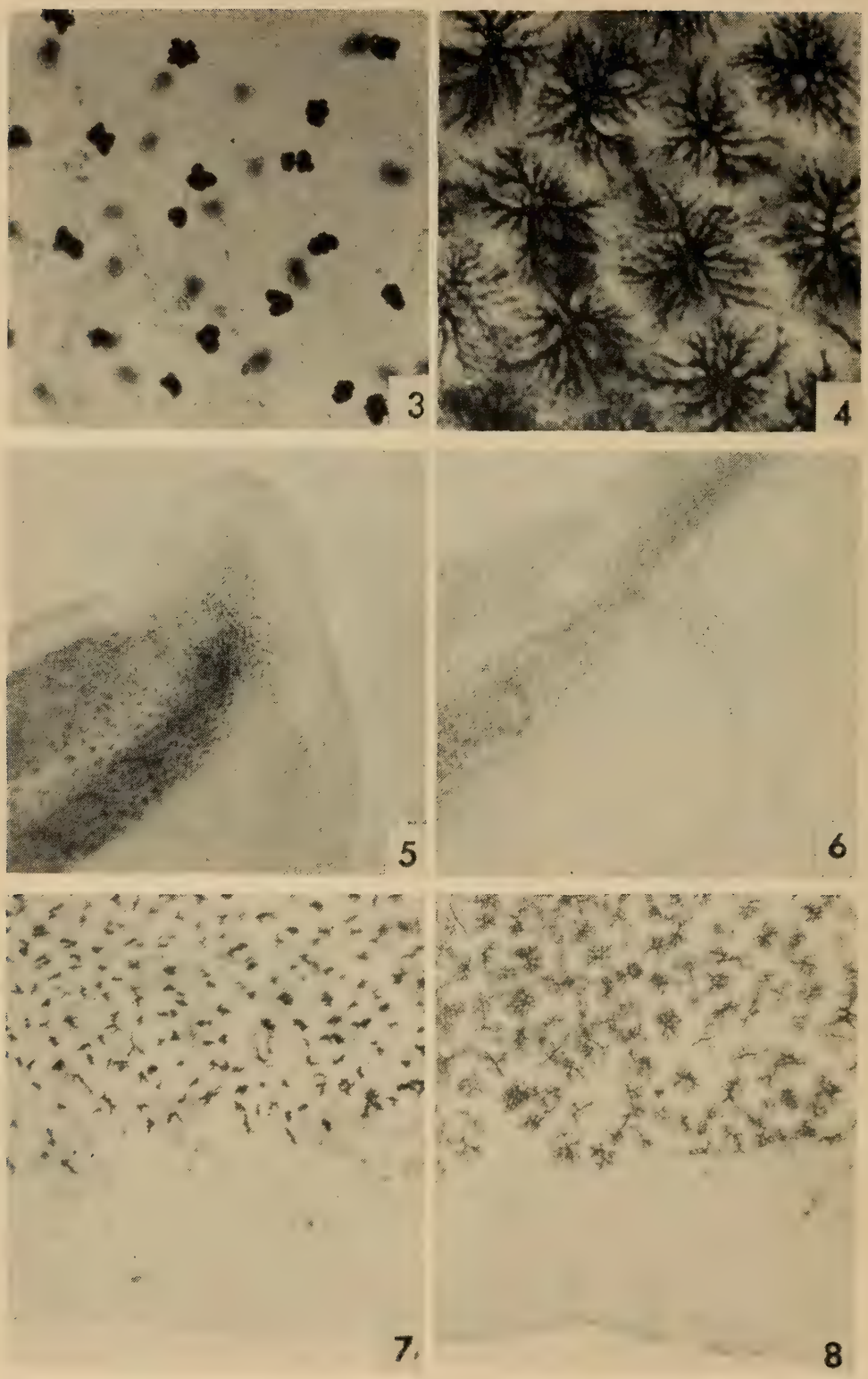

FIGURE 3. Mature melanophores of ventral fin in full contraction after bright illumination. Magnification: × 100.

FIGURE 4. Mature melanophores of ventral fin in full expansion during the tail darkening reaction. Magnification: × 100.

FIGURE 5. Fifth day of tail regeneration. Note melanophores streaming from the blastema at the line of amputation. Magnification: × 7.

FIGURE 6. Seventh day of tail regeneration. Melanophore streaming much more obvious. Magnification: × 7.

dorsal fins. At this time, the formation of melanophores begins in the new ventral fin near the base of the somitic blastema. During the next 6 or 7 days, these melanophores increase in number and appear as a stream of cells stemming from the regeneration blastema (Figs. 5 and 6). These melanophores are present only in the ventral fin and remain excluded from the area anterior to the original cut. Thus, the proximal area retains its unpigmented state. During the course of tail regeneration, tail melanophores gradually appear in the ventral fin, first making their appearance adjacent to the newly forming somites. After the tenth day of regeneration, the somitic core of the regenerating tail appears as a long spike to which is attached a generous ventral fin. Melanophores are abundant in the upper half of the fin near the developing somites, but are lacking in the lower half near the edge. After three weeks, tail regeneration is practically completed and the

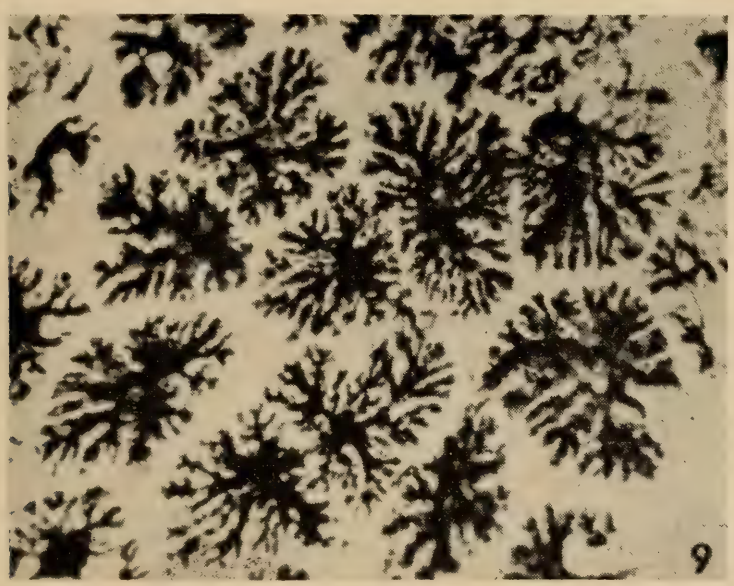

FIGURE 9. Melanophores typical of the head of mature larvae under normal illumination. Magnification: × 100.

new ventral fin is copiously supplied with melanophores. However, a clearly defined margin is present along the ventral edge of the fin (Figs. 7 and 8) which is permanently devoid of melanophores. Moreover, the melanophores at the edge of this margin are completely differentiated and there is no indication of any newly forming melanophores which might ultimately invade the clear margin. The unpigmented border extends along the complete length of the regenerated ventral fin except for the extreme proximal portion of the pigmented zone. This is the point at which the stream of melanophores was first seen in connection with the regeneration blastema. Apparently at the start of regeneration, a sufficient stimulus for melanization allows the complete reconstitution of pigmentation in this localized region. Presumably, in later stages of regeneration, the melanizing

FIGURE 7. Unpigmented area at edge of ventral fin at twenty-first day of regeneration. Slight tail darkening reaction is evident because larva was preserved immediately after removal from a murky aquarium. Magnification: × 10.

FIGURE 8. Larva similar to that in Figure 7. This larva, however, was placed in the dark before fixation; thus, the regenerated fin melanophores are fully expanded. Magnification: × 10.

stimulus is not sufficiently strong for complete repigmentation and thus the clear marginal area is formed.

Just as in normal development, the fully differentiated melanophores in the regenerating fin are light-sensitive (Figs. 7 and 8). Under normal illumination, the melanophores are punctate in appearance; but after exposures to darkness of thirty minutes or more, they expand and assume a stellate form. Differentiating melanophores in the tail regenerate are also light-sensitive just as they are during normal development (Fig. 2).

IV. Observations on other melanophores

In contrast to the melanophores of the tail fin which expand upon exposure to darkness, the large epidermal melanophores of the head (Fig. 9) contract after similar treatment. This reaction was observed empirically by Bles (1905), but no quantitative estimate of the response was recorded. In the present experiments, it was observed that the average M. I. for head melanophores of 64 tadpoles which were kept under normal illumination was 4.7, while the M. I. for 74 tadpoles fixed after exposure to darkness was 3.3. This response to changes in illumination is not as marked as that shown by the melanophores of the tail fin but it is clearly visible even macroscopically on the tadpoles which, except for the tail, appear to blanch after exposure to darkness.

Discussion

The fact that light-sensitive melanophores develop in the fin of Xenopus larvae poses several questions of embryological and physiological significance. First of all, what mechanism allows these peculiar light-sensitive melanophores to form only in the distal half of the fin, leaving the proximal portion completely free of this type of chromatophore? Stevens (1954) has shown that the posterior trunk tailfold areas of Xenopus neurulae are a good source of melanophores; thus the entire ventral fin has an adequate reservoir of melanoblasts upon which to draw. Realization of the melanophore potential is another matter, however, and may be dependent, as Twitty and Niu (1954) have suggested for urodeles, upon migratory responses of melanophores to environmental stimuli. It is possible, therefore, that the proximal area of the ventral fin does not provide an "attractive" stimulus for the migrating melanoblasts and that instead such cells migrate and complete their differentiation in the more "attractive" distal portion of the fin. Because of the peculiar melanophore distribution and because of the lack of other varieties of pigment cells in the tail, it is hard to explain the tail pigmentation patterns on the basis of physiological antagonisms between migrating chromatoblasts as has been suggested by Twitty and Niu (1954).

As an alternative explanation, it can be supposed that melanoblasts invade both the proximal and distal areas of the fin, but for reasons as yet unknown, only the distal portion of the tail provides a medium favorable for melanin synthesis. Wilde (1955) has strongly suggested that special metabolism of phenylalanine is required for normal differentiation of neural crest elements; thus, one might reason that prevalence of phenylalanine in the distal region may provide the stimulus for tail fin melanization. Furthermore, a gradient of this substance or some other melanogenic substance flowing outward from the somite to the edge

of the ventral fin would possibly account for the apparent differentiation gradient observed in the fin melanophores during normal development. Consistent with this hypothesis, the unpigmented margin along the edge of the regenerated fin may result from either a gradient which is too weak at its periphery, or from a diffusion blockage which does not allow penetration of the melanogenic substance to the edge of the fin.

Another fundamental question which suggests itself is concerned with the fact that not only are the tail melanophores extraordinarily light-sensitive but their responses are just the reverse of those of melanophores of other areas. The present observation that epidermal melanophores on the head and dorsal surface contract as a result of dark exposure is known for other amphibian larvae: for Ambystoma, Babak (1910) and Laurens (1917) and for Taricha and Rana, Bagnara (unpublished). To our knowledge, however, the very marked melanophore expansion, such as is seen in the tail of Xenopus, is unique among Amphibia. What mechanism allows the tail melanophores alone to possess this capacity is an enigma. Apparently the distal tail fin provides an environment which is favorable not only for melanogenesis but also for the development of a light-sensitive mechanism.

Whether the dorsal melanophores of Xenopus larvae are directly stimulated by light has not been determined; however, one is led to think that this is so because of the observations by Laurens (1917), who showed that the dorsal melanophores of blinded Ambystoma larvae behave toward light like those of intact animals. His additional observation that melanophore expansion after removal to light occurs much faster than the original melanophore contraction brought about by dark-treatment suggests that a photochemical reaction is involved in this system just as for the tail fin melanophores. Although light sensitivity of the dorsal melanophores is apparently a common thing among amphibian larvae, it should be emphasized that the reaction is relatively subtle, with changes of approximately 2 units of M. I. compared to a change of about 4 M. I. units for Xenopus tail melanophores.

Coincident with the unusual behavior of the tail melanophores of Xenopus is the unique form exhibited by these chromatophores. In an expanded state, their very thin branches lead one to suspect that they have less melanin than the thick melanophores on the dorsal surface. Perhaps this is a reflection of the apparent low sensitivity of the tail melanophores to the chromatotrophic hormone. Under normal lighting conditions, when the dorsal melanophores are expanded under influence of CTH, the contracted state of the tail melanophores implies that they are not sensitive to the circulating level of hormone. If the amount of hormone is raised by injection of CTH (Thing, 1952), the tail melanophores expand, strongly suggesting that the threshold level of response to CTH is higher for the tail melanophores than for those on the dorsal surface. That the tail darkening reaction is independent of hypophyseal stimulation was shown by Bagnara (1957) who pointed out that it occurs equally well in normal larvae, in hypophysioprivic larvae and in excised tails.

It is interesting to note that the response of the tail melanophores to light appears fairly early during the differentiation of these cells. Possibly it may even be present in melanoblasts before the synthesis of melanin. The reaction does not appear to be very strong in young melanophores; however, due to the relatively

small amount of melanin in these young cells, it could be fully developed and not appear so. It is significant that the melanophores of the regenerated tail can carry out the tail darkening reaction. This seems to indicate that the tail has regenerated completely, not only morphologically but physiologically as well.

Summary

1. The peculiar light-sensitive melanophores in the ventral fin of Xenopus larvae first appear about stage 47. Gradually, pigmentation increases in the distal half of the fin, but the proximal portion remains free of melanophores throughout the larval period. New melanophores first become visible at the fin edge, with an apparent gradient of melanophore differentiation from somite to edge. The young melanophores are thin and elongated, but as differentiation proceeds they add new projections and become stellate. Even the youngest melanophores exhibit some degree of tail darkening in the absence of light, but the strongest response is displayed by the fully formed melanophores.

2. Four days after tail extirpation, melanophores are seen in the regenerating ventral fin. Pigmentation returns to all of the ventral fin except for a margin along the fin edge, with a "somite-to-edge" gradient again apparent. The new melanophores are of the typical tail fin type and are fully light-sensitive.

3. Melanophores on the dorsal surface differ markedly from those on the tail. The former are heavily pigmented and are apparently more sensitive to the chromatotrophic hormone than the latter. Dorsal melanophores contract upon exposure to darkness and expand in the light. They are less reactive to changes in illumination than the tail fin melanophores.

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